

Evaluation of a liquid culture system in the detection of mycobacteria at an antituberculosis institution in China; A retrospective study

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Abstract

Objective: A retrospective study comparing the diagnostic performance of the BACTECTM MGITTM 960 system (M960 system; BD Worldwide, Franklin Lakes, NJ, USA) with Löwenstein–Jensen (L–J) culture to detect mycobacteria in sputum specimens from patients with suspected pulmonary tuberculosis (TB).

Methods: Sputum samples were analysed for the presence of acid-fast bacilli (AFB). Samples were inoculated into the M960 system and L–J culture. Positive cultures were examined for the presence of AFB.

Results: The M960 method detected significantly more positive samples than L–J culture (818/1676 [48.8%] vs 692/1676 [41.3%]). Using L–J culture as reference, the sensitivity, specificity, positive predictive and negative predictive values of the M960 system were 91.0%, 76.1%, 77.0% and 92.2%, respectively. The time-to-detection of mycobacteria was 11.78 ± 5.16 days for M960 and 24.17 ± 8.73 days for L–J.

Conclusions: The M960 system had better diagnostic capability than L–J culture. Clinical value may be maximized by combining results from both methods.

Keywords

Liquid culture, solid culture, mycobacteria, tuberculosis, BACTEC MGIT 960, Lowenstein–Jensen

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Introduction

Tuberculosis (TB) is a deadly infectious disease caused by *Mycobacterium tuberculosis*. The worldwide prevalence of TB was 9.6 million in 2014, with 1.5 million deaths.¹ China has the third highest number of incident and fatal cases of TB worldwide (930 000 and 38 000, respectively), accounting for 9.7% of cases.¹

Fast and effective microbiological diagnosis is essential to control the spread of TB. The most common diagnostic method is sputum smear microscopy,² with culture methods or rapid molecular testing used in countries with advanced laboratory facilities.² In China, antituberculosis institutions and some general hospitals usually rely on smear tests and radiographic evidence to diagnose TB.³ Although these traditional methods remain the cornerstone of diagnosis, their sensitivity is low and variable.^{4,5} The use of liquid medium culture methods with automated incubation and reading systems (e.g. MB/BacT ALERT[®] 3D system [BioMérieux; Marcy-l'Étoile, France] or BACTEC[™] MGIT[™] 960 system [BD Worldwide; Franklin Lakes, NJ, USA]) have been shown to improve the isolation of mycobacteria from clinical specimens.^{6,7} The BACTEC[™] MGIT[™] 960 (M960) system comprises plastic tubes containing Middlebrook 7H9 medium with a fluorescent growth indicator embedded in silicone at the bottom of each tube. This indicator is sensitive to the concentration of oxygen in the broth medium. Clinical specimens are processed and inoculated into the M960 tubes. As micro-organisms grow, the oxygen in the medium is consumed with a subsequent increase in the fluorescence of the indicator. The fluorescence in tubes is monitored automatically every 60 minutes using a photodetector.

The aim of this retrospective study was to compare the diagnostic performance of a liquid medium culture method using the M960 system with the solid

Löwenstein–Jensen (L–J) culture method to detect mycobacteria in sputum specimens from patients with suspected pulmonary TB.

Patients and methods

Study population

This retrospective analysis included data collected between 1 February 2013 and 20 June 2014 at the Tuberculosis Clinic, Chaoyang District Centre for Disease Control and Prevention, Beijing, China. Sputum samples from presumptive cases of pulmonary TB were collected by the same investigator (Q.Y.) before the patients had received any treatment.

The study was approved by the ethics committee of Chaoyang District Centre for Disease Control, and all patients provided written informed consent.

Sample analysis

Smears were prepared from all specimens and examined for acid-fast bacilli (AFB). In brief, smears were inactivated by ultraviolet radiation for 2 h in a class II biosafety cabinet, then treated with Ziehl–Neelsen (Z–N) stain and examined via light microscopy.

Sputum samples were digested and decontaminated using an equal volume of sodium hydroxide (final concentration 1%) and *N*-acetyl-L-cysteine (final concentration 0.25%) for 15 min.⁸ After neutralization with phosphate buffer, 0.5 ml of decontaminated suspension was inoculated into M960 culture tubes containing 0.8 ml manufacturer's growth supplement and antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin [PANTA]). The tubes were placed into the BACTEC[™] MGIT[™] 960 instrument, where they were incubated at 37°C and monitored automatically every 60 min for 6 weeks or until mycobacterial colonies were detected. In addition, 0.1 ml of the remaining suspension was inoculated onto L–J

medium and incubated at 37°C with examinations at least once a week for 8 weeks or until mycobacterial colonies were detected.

Specimens were considered negative if no mycobacterial growth was detected after 6 weeks (M960 system) or 8 weeks (L–J medium). Smears were prepared of all positive specimens. If staining confirmed the presence of AFB the culture was defined as positive, and if staining found other Gram-negative bacteria but no AFB then the culture was considered contaminated.

Statistical analyses

Data were presented as mean ± SD (range) or *n* (%). The recovery and contamination rates of the two systems were compared using χ^2 test. Student’s *t*-test was used to compare the time-to-detection (TTD) in different media. *P*-values < 0.05 were considered to be statistically significant and statistical analyses were performed using SPSS® version 13.0 (SPSS Inc., Chicago, IL, USA) for Windows®.

Results

The study included a total of 1676 samples from patients with presumptive pulmonary tuberculosis. Data regarding sputum AFB findings and culture results are shown in Table 1. In total, 506/1676 (30.2%) of

sputum smears were AFB positive. Significantly more AFB-negative samples were found to be mycobacteria-positive following M960 culture than L–J culture (322/1170 vs 200/1170; *P* < 0.01). There was no significant difference in the number of AFB-positive samples found to be mycobacteria-positive following M960 culture or L–J culture. Contamination rates were significantly higher for M960 culture than L–J culture (84/1676 [5.0%] vs 46/1676 [2.7%]; *P* < 0.01). When data from the L–J and M960 cultures were combined, the positivity of presumptive TB cases increased significantly from 48.8% (818/1676; M960) and 41.3% 692/1676; L–J) to 52.5% (*P* < 0.05 for each comparison; *n* = 1676).

Table 2 shows the number of positive samples detected by the M960 and L–J methods and their subsequent AFB smear findings. The M960 method detected significantly more positive samples than the L–J method (818 vs 692; *P* < 0.01). The proportion of isolates that were identified positive by either culture and then subsequently found to be negative or contaminated was 21.4% ([180 + 8]/880) for the M960 system and 9.0% ([47 + 15]/692) for L–J culture (*P* < 0.01).

Using L–J culture as the reference method, the M960 system had 91.0% (630/692) sensitivity, 76.1% (714/938) specificity, 77.0% (630/818) positive predictive value (PPV),

Table 1. The presence or absence of acid-fast bacilli (AFB) in sputum samples from presumptive cases of pulmonary tuberculosis, and related findings of cultures for mycobacteria using the BACTEC™ MGIT™ 960 system (BD Worldwide; Franklin Lakes, NJ, USA) or Lowenstein–Jenson method (*n* = 1676).

| Sputum AFB finding | BACTEC™ MGIT™ 960 system | | | Lowenstein–Jenson method | | | Total |
|--------------------|----------------------------|----------------------------|-------------------------------|----------------------------|----------------------------|-------------------------------|-------------|
| | Positive <i>n</i> = 818 | Negative <i>n</i> = 774 | Contaminated <i>n</i> = 84 | Positive <i>n</i> = 692 | Negative <i>n</i> = 938 | Contaminated <i>n</i> = 46 | |
| Positive | 496 (60.6) | 4 (0.5) | 6 (7.1) | 492 (71.1) | 9 (1.0) | 5 (10.9) | 506 (30.2) |
| Negative | 322 (39.4) | 770 (99.4) | 78 (92.9) | 200 (28.9) | 929 (99.0) | 41 (89.1) | 1170 (69.9) |

Data presented as *n* (%)

Table 2. Findings of cultures for mycobacteria in sputum of patients with presumptive pulmonary tuberculosis, using the BACTEC™ MGIT™ 960 system (BD Worldwide; Franklin Lakes, NJ, USA) or Lowenstein–Jenson method, and the presence or absence of acid-fast bacilli (AFB) in post-culture samples (*n* = 1676).

| | | Lowenstein–Jenson method | | | |
|-----------------------------|--------------|--------------------------|--------------|--------------|-------|
| | | AFB positive | AFB negative | Contaminated | Total |
| BACTEC™ MGIT™ 960 system | AFB positive | 630 | 180 | 8 | 818 |
| | AFB negative | 47 | 714 | 13 | 774 |
| | Contaminated | 15 | 44 | 25 | 84 |
| | Total | 692 | 938 | 46 | 1676 |

Data presented as *n*

and 92.2% (714/774) negative predictive value (NPV) for the recovery of mycobacteria (Table 1).

The mean TTD was significantly shorter for the M960 system than for L–J culture (11.78 ± 5.16 days [range 4–33 days] vs 24.17 ± 8.73 days [range 11–69 days]; $P < 0.01$).

Discussion

The results of this study showed that the M960 system provided a better isolation rate of mycobacteria from sputum specimens from patients with suspected pulmonary TB than L–J culture. Our findings are similar to those from other studies in several different countries that have evaluated the same culture methods.^{4,7,9–12} Culture using solid media plays an important role in the isolation of mycobacteria from sputum specimens, and current international guidelines recommended the use of one solid medium and one liquid medium.¹³ Combining data from the L–J and MGIT media (M960 system) in the present study significantly increased the number of positive culture findings.

In China, TB is usually diagnosed via X-radiography and smear staining.^{3,14} The detection rate by X-radiography is low, at approximately 33.8%¹⁵ and the average rate

of observer under-reading and over-reading of X-radiographs can reach 21.8% and 19.5%, respectively.¹⁶ Chest X-radiography is useful but is not sufficiently specific to diagnose pulmonary TB, and the disease can easily be misdiagnosed especially in smear-negative cases.¹⁵ The M960 system was effective in the identification of TB in the present study, particularly in smear negative cases. In addition, the TTD was significantly shorter with the M960 system than L–J culture. TTD is an important feature of diagnosis since early identification allows timely treatment and can prevent the disease from spreading. The continuous monitoring feature of the M960 system has an unequivocal advantage over the weekly examination of L–J cultures.

It is inevitable that a small proportion of cultures will be contaminated by other organisms. As a general rule, a contamination rate of 2–3% is acceptable in laboratories that receive fresh specimens,¹⁷ and the Chinese Antituberculosis Association suggests the contamination rate should be controlled at 2 – 5%.¹⁴ Contamination was not a serious problem in the present study, and was less common than in many other studies.^{4,7,9–12} The rate of contamination was higher for M960 than for L–J culture, but this may be related to the fact that the medium in the M960 system is

more enriched than L–J medium. A review of the literature found a large variation in contamination rates for both media.¹⁷ These wide variations may reflect differences in quality of specimens, collection, transport and testing times.

We have shown that the M960 system can significantly increase the number of TB cases identified and allow earlier detection of mycobacteria compared with the traditional L–J method, and so can assist in the definitive diagnosis of presumptive TB. However, a major drawback of the M960 system is its expense, with the average cost per test (including tube and reagents) approximately 5 – 6 times more than L–J culture. Nevertheless, the sensitivity and rapidity of the M960 system are major advantages over the traditional L–J method and may bring benefits not only for the individual patient but also for the community by limiting the disease and its transmission.⁴

The present study is limited by the fact that positive cultures were not characterised, so it was not possible to distinguish between *Mycobacterium tuberculosis* and non-tuberculosis mycobacteria (NTM). In addition, only one liquid medium culture method was evaluated and molecular biology methods were not used. Therefore, further studies are required.

In conclusion, the M960 system was shown to be a sensitive, rapid and fully automated instrument for the recovery of mycobacteria from sputum specimens. Its clinical value may be maximized by combining results with those from the traditional L–J solid media culture method.

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Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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